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A New Method in Walking Analysis Using the Angles around the Midpoint between Print Length and Toe Spread by Four Different Color Footprints

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Abstract

Sciatic function index (SFI) has long been used as one of the evaluation methods of walking analysis. SFI is a method in assessing nerve function through footprints in walking analysis. Methods that previously have been developed use one color ink, but this method is difficult to interpret. The interpretation is difficult because of mingled footprints among the mice four legs. If rats do not walk straight, their footprints will be overlapped to one another and complicate the measurement. SFI is a method using a special mathematic formula. Many researchers questioning this method because this method is affected by the muscle contracture, auto mutilation and the way and speed of the rat walk.

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In this study we use four different colors, which allow us to more easily interpret the results of the footprint. In this study, we modify a tool from the existing tools in other literatures. The measurement result of this tool is print length (PL), inter toe spread (ITS) and toe spread (TS), which belong to the SFI component. This tool must be valid and reliable when it is used to measure the components of measurements in the function analysis of the nerves. Validity and reliability are needed to ensure that research data can be trusted. We confirm the SFI result with the toe out of angle (TOA) and a new method to describe the normal footprint. TOA is believed to be the important measurement in walking analysis especially it affects the change of abduction-adduction and internal-external rotation of hind limb. The purpose of this study was to develop a new method to assess the nerve functional analysis, not only using measurement of the SFI and TOA, but also the angles around the meeting point of PL and TS. This study was a laboratory-based research. The subjects of the research are 6 Sprague Dawley rats. We use student t test to analyze the TOA data of the right and left hind limb. Correlation study is applied in analysing the result of SFI, TOA and the angles around the meeting point of PL and TS. There is no significant difference between the right and left hind limb. There is a good correlation between SFI, TOA and the angles around the meeting point of PL and TS. We conclude that the angles around the meeting point of PL and TS can be used as an additional new method in walking analysis to confirm the result of SFI and TOA.

Keywords: walking analysis; angles around the midpoint between print length and toe spread; four different colors footprint

1. Introduction

The quite common peripheral nerve injury, as reported in a study, is suffered by 3-10% of patients [1, 2, 3]. Peripheral nerve injury therapy requires a huge cost and a long duration of hospitalization [4, 5]. Moreover, the target of nerve injury healing, which is the recovery of nerve functions, is still uncertain; hence, studies on acute peripheral nerve injury therapy need to be conducted [5, 6]. Peripheral nerve injury causes disability in young adult male community, which members are mostly part of the working age group, therefore it is important to study it. Nerve injury requires long duration of outpatient care and recovery is influenced by several factors [5, 6].

Studies on peripheral nerve injury therapy mainly in sciatic nerve, tibial nerve, and common fibular nerve injuries usually use animals to discover the effect of therapy. Axotomy of sciatic nerve implemented by complete transection at mid-thigh of rats or mice is the oldest animal experimental model of peripheral nerve injury [7]. A certain parameter is needed to measure the effect of therapy given. Standardized measurements should be able to measure what should be measured and should be reliable. Measurements can be performed using certain instruments. Research instruments can be in the form of a specific tool or a special instrument such as a questionnaire or a particular tool [8].

To assess the clinical progress of peripheral nerve injury in experimental animals the nerve function indexes are determined. Research instruments that are used in sciatic, tibial, and fibular nerve function indexes are the footprints of walking rats. Rats' footprints are very difficult to analyze if the rats are allowed to walk without guidance. The basic movements of rats' hind limb are abduction, adduction, extension, flexion and rotation. The movements of rats' hind limb are influenced by the body posture during walking, whether the rats are walking straight or in zig-zag manner. Vareja˜o et al. described in their study the existence of a good correlation between Toe Out Angle (TOA) and SFI during the healing process of sciatic nerve injury. TOA explains the biomechanic consequences of stance phase especially the external rotation movement in the walking mechanism of rats. The angle changes of TOA can occur because the lateral and medial parts of the paw that are innervated by tibial and fibular nerve are branched from sciatic nerve can be influenced during injury [9].

The validation of sciatic nerve injury recovery evaluation measurements in rats that are widely used has been questioned by many researchers. These researchers consider the influence of muscular contracture, auto mutilation, speed and direction of walking against SFI measurement results [10,11,12].

Thus, a question arises on whether the developed parameter is quite consistent and has positive correlation between several parameters. Therefore, this research will study an additional paremeter, i.e. the angles formed by two intersecting lines, which are the line between the heel and the third toe and the line between the thumb and the little toe. The angles formed have not been analysed yet in the walking pattern of rats, but it is possible that these angles are important to explain the effect of abduction-adduction movements, the external-internal rotation of rats' hind limbs and the possibility of being directly proportional to others functional parameters.

2. Research Methods

This study has been approved by the Ethical Commission of the Faculty of Medicine, University of Indonesia, ethical clearance number is 482/H2.F1/ETK/2014. The study used 6 Sprague Dawley rats as experimental animals. The animals were obtained from the Animal Laboratory of the Department of Histology of the Faculty of Medicine. The rats were randomly selected and weighing between 200-300g. The examination of nerve function index was performed by inserting the rats into an alley. Before the rats enter the alley [9], the alley was covered by graph paper with certain length and size that had been optimized.

Before the examination, the four paw of rats were given four different colors, i.e. red, green, purple and black. The rats were left walking inside an acrylic alley with specific size. When walking, the rats would step on the paper used to cover the alley's floor, so the rats' footprints could be seen clearly on the graph paper.

TOA was examined by measuring the angles between the line from the heel toward the toe against the reference line between the heel and the tip of the third toe. Length measurements were conducted on several lines; the first was the distance between the heel and the tip of the third toe (PL), the second was on the line between the tips of the thumb and the little toe (IT), and the third was the the distance between the tips of the second and fourth fingers (TS). Each was measured on the right and left foot of rats' hind limbs during straight walking [13, 14].

The angles between two intersecting lines would be measured on the angle formed between PL and TS; to simplify we would call them quandrant 1, 2, 3 and 4. Each quadrant would be measured on the right and left paw of rats' hind limbs.

In each walking cycle, each parameter was measured on five footprints. The measurement results of the five footprints were calculated to obtain the mean value. The results of PL, IT and TS measurements were used to compute SFI, TFI, and PFI data. The functional index and angles' mean value (TOA, Q1, Q2, Q3 dan Q4) that had been decided were analysed using SpSS software through normality test. Correlation test between the parameters of nerve function indexes was analysed using a test in accordance to the result of normality test. The footprints' midpoint needs to be determined; the foot's midpont was the intersection of PL and TS lines.

Q1: the angle between the line from the midpoint to the tip of the third toe and the line from the midpoint to the tip of the thumb

Q2: the angle betwen the line from the midpoint to the thumb and the line from the midpoint to the heel

Q3: the angle between the line from the midpoint to the heel and the line from the midpoint to the tip of the little toe

Q4: the angle between the line from the midpoint to the tip of the little toe and the line from the midpoint to the tip of the third finger

The data obtained would be tested with t test to determine the existence of significant difference between the first and the second measurements.

3. Research Results

Before the rats were tested with the walking tract, each of their weights were measured using an analytical scale weight (g) and recorded as in Table 1.

Table 1: Rats' Weights

Rat	Weight (g)
1	200
2	210
3	220
4	200
5	210
6	223

This study applied repeated measurements and found that t test showed no significant difference between the first and second measurements. Repeated measurements were done on the same subjects, in similar condition, and the measurement tool showed consistent results.

Kolmogorof Smirnov test showed that the data had normal distribution (p> 0.05). SFI and TOA data in the form of numeric scale data were tested using Pearson Correlation test and the result showed good correlation. The data of the four angles surrounding the meeting point between PL and TS were found to have no significant difference in the repeated measurements on normal rats' footprints.

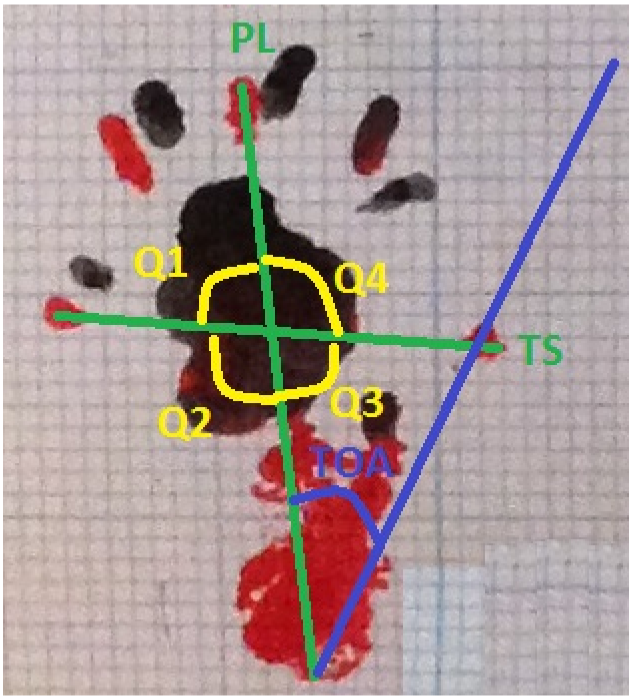

Figure 1: The measurement result of PL, TS, TOA, Q1, Q2, Q3 and Q4 of the left foot

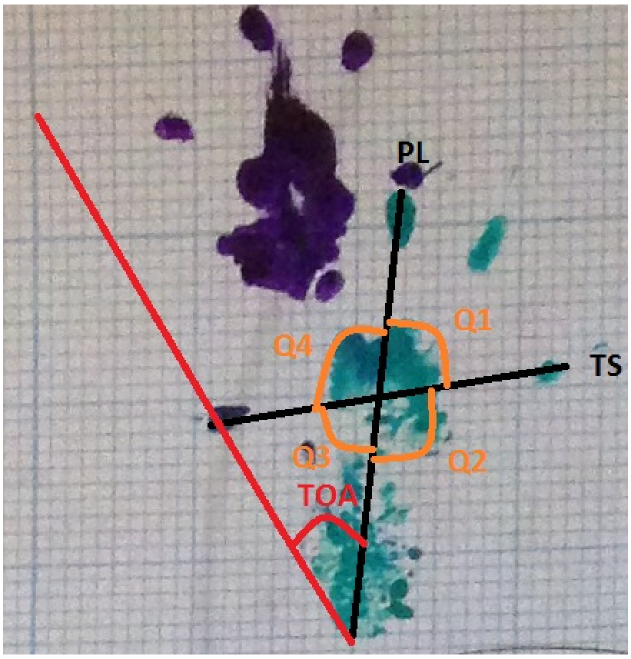

Figure 2: The measurement result of PL, TS, TOA, Q1, Q2, Q3 and Q4 of the right foot

4. Discussion

The most common method used in nerve function evaluation after nerve injury on rats is footprint analysis. Rats' footprints were obtained from the touch of toruli digitales toe I to V on the measurement field. Measurement field is an area covered by graph paper to print the footprints so that they can be measured. The measurements of PL, IT and TS can be used to analyze nerve function or known as nerve function index. This nerve function index can analyze the nerve functions that are important in regulating the walking function, i.e. sciatic, fibular, and tibial nerve functions. Sciatic Function Index (SFI) was developed by De Medinaceli, and later on adapted by Bain [13, 14].

Walking is a systematically regulated reflect that involves many organs, such as extremity muscles and torso, and also the coordination of various joints. Several literatures mention the existence of nerve network structure in the spinal cord (*medulla spinalis*) that has a role in regulating locomotion, which can work even though there is no regulation from the central nervous system. This neural network is actually an interconnected nerve network called Central Pattern generator (CPG). Stimulation in the spinal cord at CPG will cause movement without passing through the central nervous system or commonly known as reflex. The proof of CPGs in humans is still being studied, although several comprehensive clinical studies from researches on animals and humans have studied the mechanism of CPG in regulating locomotion or movement [9, 10, 11].

The central nervous system (CNS) works by coordinating simultaneously when an organ should be moved, how far and the time of movement. A movement can only be performed correctly if a series of biomechanic requirements is met. A movement uses electrical signal pattern that is sent along the nerves to activate the correct muscle cells. The sciatic nerve in rats mainly (98-99%) comes from the spinal cord at the level of L4 and L5; however, there are reports stated that the sciatic nerve comes from L3 and L6. Some reports mentioned that the sciatic nerve in rats come from the lumbosacral branch of L4-S4 from the spinal cord.

Before using the SFI alley and the four color inks, rats were examined by letting the rats walk after being marked with red ink. The first examination result can be seen in Figure 1. It is not clear from the figure whether the footprints produced were from the right or left paw or whether they were from the front or hind limb. In Figure 2, footprints with irregular direction can be seen; the rats tended to walk without direction, so the distance between fingerprints were difficult to measure.

The use of SFI alley helped rats to walk straight; walking straight is an important measurement to examine nerve functions. Straight walking is more preferred to examine sciatic nerve function because the data obtained are not influenced by the rats' torso movement and by the influence of torso movement on orthogonal distance from toe I to foot one to toe I of different foot (TOF). TOF can be used to analyze sciatic nerve function by using the SFI formula that is different from that of De Medinaceli, Freed, and Wyatt [10].

The rat has very well developed gluteus superficialis, biceps femoris, semitendinosus and gastrocnemius hip muscles, which are even more developed than humans. Gluteus superficialis, biceps femoris and semitendinosus muscles are located at upper hind limb. Gastrocnemius muscle is located in lower hind limb.

Figure 3: The measurement result of rat's footprints without using SFI alley and with one ink color

Figure 4: Rat's footprints without direction

Theoretically, in rats' torso movement, especially in sagittal plane, there are two main typical movement according to its distance to sagittal plane, i. e. abduction and adduction. The important muscles in abduction of hind limb are superficial gluteal and biceps femoris muscle. Superficial gluteal muscle originates in the os ilium and inserts in femur. The biceps femoris muscle has an *origo* in vertebrae and insertion in the tip of distal femur and proximal tibia. When both muscles abduct the hind limb, not only the two muscles are needed as the prime mover, but also the gastrocnemius muscle in lower hind limb. Gastrocnemius muscle known as the hamstring muscle attaches to Achilles tendon and os calcaneus. These muscles are innervated by the tibial nerve that also innervates plantaris muscle. During the abduction, the movements of these muscles cause a change in rat's torso position. The change in rat's torso movement may cause a change in the angle between the line that connects the heel and the third toe, called the inter phalanx length against the line that connects toe I and toe V, called the toe spread. The angle between the two lines is rarely measured, and is not used in analyzing sciatic nerve function.

This study uses SFI alley to ensure that the rats walk straight as seen in Figure 3. Figure 3 shows the unmarked rats' paw. Although rats have been ascertained to walk straight to determine the sciatic nerve function index, the analysis was still difficult. First, the rats walked very fast, causing in-optimal data recording and collecting. Ink markers with different colors were needed for each foot and the floor had to be covered with graph paper. The objective of the use of different four was to ensure that the rats' footprints could be easily analyzed and studied in detail as seen in Figure 7A and 7B, which showed the paper with rats' footprints.

Figure 5: A rat walking in SFI alley

Sciatic nerve passes the deep part of biceps femoris and superficial gluteal muscle. Sciatic nerve branches into tibial, fibular and sural nerve. Each nerve innervates the muscular groups, which function to move the lower limbs of rats.

Biceps femoris muscle is a member of superficial muscle group of rat's hind limbs. Biceps femoris muscle is located on the side of rat's thigh and posterior toward gluteal area and consists of two caputs. Biceps femoris muscle serves to function in upper limb's abduction movement. Biceps femoris muscle has three caputs, which have origos in sacrum and caudal vertebrae. Biceps femoris muscle has insertion in distal caput femoris and 2/3 of proximal tibia. This muscle is mainly innervated by fibular nerve that comes from the sciatic nerve, so the injury occurred in sciatic nerve will influence this muscle's motoric movement [16, 17].

Figure 6: A rat walking in SFI alley. The paw were painted using four ink colors

Figure 7A: A paper showing rat's footprint in four colors without alley; **Figure 7B:** A paper showing rat's footprint in four colors with alley

The tibial nerve innervates the tibialis anterior muscle that functions as the main motor of the dorsiflexion movement of rat's heels. The weak performance of this muscle will influence the walking movement of rats. Tibial nerve also regulates the muscle movements of *gastrocnemius caput lateral*e and *caput mediale*. In plantaris muscle, tibial nerve branches in two, namely medial plantar nerve (*nervous plantaris medialis*) and lateral plantar nerve (*nervus plantaris lateralis*). Medial plantar nerve that is located at the medial part of rats' paw innervates the muscles in the medial plantar side. Lateral plantar nerve regulates muscle movements in the lateral section of the paw. Compared to humans, rats have a slight different neural topography.

The neural topography of rats, as reported by Decosterd and Woolf in 2000 (Figure 8), shows that sciatic nerve branches into three main nerves, i.e. *nervus peroneus communis, tibialis anterior*, and *suralis*. TOA in Figure 9 measures the angles that involve the medial side of rat's footprints. The medial side (nearing toe I/toe thumb) of dorsal and plantar sides of rat's hind limb is only innervated by saphenous nerve; therefore, sciatic nerve injury will possibly cause no changes because saphenous nerve is not branched from sciatic nerve, but comes directly from the fasciculi of spinal cord as high as L3. Sciatic nerve injury that affects *nervus peroneus communis, tibialis anterior*, and *suralis* will lead to dropout toward healthier side, which is the medial side; hence, it will influence the width of TOA.

Lateral and middle sides of the hind limb are innervated by *nervus peroneus communis, tibialis anterior*, and *suralis,* which are originated from sciatic nerve. If the sciatic nerve is injured, there will be possibility that the three nerves will be impaired. The innervation area was explained by Swett and Woolf research in 1985 [15]. This idea leads us to analyse the profiles of Q1, Q2, Q3 and Q4 angles. The four angles that cover all area of the paw need to be analyzed to obtain a reference value. This value then needs to be measured further in pathological condition to find out a cut off value, which can be used as the reference to assess the angle ranges of Q1, Q2, Q3 and Q4 of normal and pathological hind limb. Clinical and epidemiological studies have identified dorsiflexion movement and heel rigidity increase is important in determining pathological condition of limb [19].

5. Conclusion

The study concludes that the analysis results of PL, ITS and TS on several repeated measurement did not show significant differences. The examination ressults of TOA, Q1, Q2, Q3 and Q4 showed consistent results, and therefore might be used as references to compare normal to sciatic nerve injured hind limb. This suggestion is based on the good correlation between the SFI with TOA, Q1, Q2, Q3 and Q4.

The results obtained in this study might complement and confirm the previous examination methods and provide additional normal profiles of rats' footprints during straight walking.

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